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A Comparative Study on Mating Behaviour between the B Biotype and a non-B Biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Zhejiang, China

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The objective of this study was to derive a description of the mating behaviour of the whitefly Bemisia tabaci*, and to compare this behaviour between the notorious invasive B biotype and a native non-B biotype of the insect from Zhejiang, China. We first did a crossing experiment between the two biotypes, and then conducted observations on the various components of their mating behaviour by continuous recording with video cameras. Reciprocal crossing trials between the two biotypes resulted in no female progeny and thus demonstrated no compatibility in reproduction between them. The whole process of mating could be described as three consecutive phases: male search for female, courtship, and copulation. In both biotypes, successful mating could be completed as early as 4–6 h after emergence and over half the individuals completed their first mating in the first 12 h. The frequency of mating in the B biotype was marginally higher than that in ZHJ1. Moreover, the frequency of mating by females in the B biotype increased by nearly three times when an extra male of the same biotype was added, while addition of an extra male in the ZHJ1 did not result in an increase of mating frequency. These results indicate that individuals of the B biotype have a stronger propensity to mate than that of ZHJ1when the proportion of males in a colony is increased. The stronger propensity and ability for mating in the B biotype may be an*

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advantage in its reproductive competition and interference with non-B biotypes of this insect.

KEY WORDS: *Bemisia tabaci*; mating behaviour; courtship; copulation; biotype.

INTRODUCTION

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a widely distributed species throughout the tropical and subtropical regions of the world. In the past two decades, *B. tabaci* has become a major crop pest on all continents except Antarctica (Brown *et al.*, 1995). *Bemisia tabaci* is a genetically diverse group (Frohlich *et al.*, 1999; De Barro *et al.*, 2000). Recently De Barro *et al.* (2005) used ITS1 and CO1 nucleotide sequences to estimate a global phylogeny of *B. tabaci* and recommended the grouping of six races for *B. tabaci*. Since then, De Barro (2005) has shown that *B. tabaci* from the Asia-Pacific region can be divided into up to 10 different genetic groups.

Some of the genetic groups of *B. tabaci* have been shown to differ in biological characteristics, such as host range, the ability to transmit viruses, the capacity to induce specific phytotoxic response, and feeding behaviour (e.g., Bedford *et al.*, 1994; Brown *et al.*, 1995; Jiang *et al.*, 1999; Lisha *et al.*, 2003; Zang *et al.*, 2005a). A range of mating experiments between populations or biotypes has shown that while some inter-biotype matings are incapable of producing female offspring others are (Perring *et al.* 1993; Bedford *et al.*, 1994; De Barro *et al.*, 2005). However, the production of female offspring via mating between populations or biotypes, an indication of reproductive compatibility, has been observed only between populations within the same race as defined by De Barro *et al.* (2005). Some observations have been made on the reproductive behaviour of *B. tabaci*. Li *et al.* (1989) provided a general description of the courtship and mating behaviour of *B. tabaci* biotype A (see Perring and Symmes, 2006, for information on the biotype identity in Li *et al.*, 1989). Perring and Symmes (2006) provided detailed descriptions of mating behaviour of the B biotype (sometimes referred to as *Bemisia argentifolii* (Bellows and Perring) in the literature) and compared behaviour of the B biotype they observed with that of the A biotype as reported by Li *et al.* (1989). In experimental studies of competition between biotypes, mating behaviour has often been briefly noted (De Barro and Hart, 2000; Pascual and Callejas, 2004). Because mating behaviour of whiteflies may change under different conditions (Byrne and Bellows, 1991), comparative observations under common conditions may be required to reveal differences between biotypes of *B. tabaci*.

The presence of *B. tabaci* in China was first recorded in 1949 (Zhou, 1949), but its importance in agriculture was virtually unnoticed until the mid 1990s. Since then, this insect has been observed to occur in high density and cause serious damage to cotton, most vegetable crops and some ornamental plants in more than 20 provinces in China (Luo and Zhang, 2000; Ren *et al.*, 2001; Liu *et al.*, 2005). Evidence has indicated that the invasive alien B biotype of *B. tabaci* has been responsible for the damage in recent years (Luo *et al.*, 2002; Qiu *et al.*, 2003; Zhang *et al.*, 2005). This biotype originated in the Middle East/Asia minor (Frohlich *et al.*, 1999; De Barro *et al.*, 2000) and has recently spread widely (Brown *et al.*, 1995; De Barro *et al.*, 2005).

Zang *et al.* (2005b) demonstrate that the B biotype of *B. tabaci* has the capacity to displace a native non-B biotype, named China-ZHJ1 population, from Zhejiang, China, in a short period of time. The ZHJ1 population belongs to the unresolved Asia group of *B. tabaci* as defined in De Barro *et al.* (2005). As part of an attempt to understand the mechanisms responsible for the invasion and displacement of native non-B biotypes by the B biotype, observations have been made on the mating behaviour of the B and non-B biotypes as well as the mating interactions and interference between the biotypes. Here, we demonstrate the reproductive incompatibility between the B biotype and ZHJ1 population, and then describe and compare the mating behaviour of these two biotypes.

MATERIALS AND METHODS

Whitefly Cultures and Adult Collection

The B biotype and a non-B population (ZHJ1) of *B. tabaci* were first collected in 2003 from cabbage and cotton in Zhejiang, China, respectively. The B biotype has been shown to be exotic to China, and ZHJ1 an indigenous population (Zang, 2005; Zhang *et al.*, 2005). A culture of each of the two biotypes was maintained on cotton (*Gossypium hirsutum* cv. Chuanmian 109) in a separate chamber set to $28 \pm 1^{\circ}$C, a 14h:10h (L:D) photoperiod, and $70 \pm 10\%$ relative humidity (All experiments and observations were also conducted under these climatic conditions). The cotton plants were cultivated in a potting mix in 2-liter pots in screen houses or climatic chambers. The same cultivar of cotton was used for all experiments and observations. The purity of the two cultures was regularly monitored using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique (Zang *et al.*, 2005b).

Fifteen to eighteen hours prior to experiments, plant leaves with whitefly pupae (late fourth instar nymphs with red eyes) were collected from each

Table I. Number of F1 Progeny and Proportion of Females in the Progeny Obtained from Crosses between the B Biotype and ZHJ1 of *Bemisia tabaci* over a 5 Day Oviposition Period

Crosses	Treatments[a]	Mean no. (±SE) of progeny		Mean proportion (±SE) of ♀ in F1[b]
		♀	♂	
Crosses within B biotype	1 ♀ × 1 ♂	9.9 ± 1.3	12.6 ± 1.3	0.44 ± 0.03 b
	1 ♀ × 2 ♂	21.5 ± 1.7	7.7 ± 0.7	0.74 ± 0.02 a
Crosses within ZHJ1	1 ♀ × 1 ♂	9.8 ± 1.5	11.3 ± 1.3	0.45 ± 0.04 b
	1 ♀ × 2 ♂	18.5 ± 1.3	19.0 ± 1.5	0.49 ± 0.02 b
Crosses between biotypes	1 B ♀ × 2 ZHJ1 ♂	0	23.6 ± 2.7	0
	1 ZHJ1 ♀ × 2 B ♂	0	33.9 ± 3.2	0

[a] Twenty replicates in each treatment.
[b] Means in this column followed by the same letters do not differ significantly ($P > 0.05$). Data of the two inter-biotype crosses were not included in the ANOVA because of lack of female progeny in these treatments.

culture and placed in 9 cm plastic Petri dishes. Preliminary observations showed that *B. tabaci* adults would not mate up to 2 h after emergence. Therefore, all newly-eclosed adults were collected 0–2 h after emergence and placed singly into glass vials for the crossing experiments and observations on mating behaviour.

Crossing Experiments

Six treatments (Table I) were conducted using clip-cages placed on leaves of cotton plants, and there were 20 replicates for each treatment. The ventilated clip-cage was made from a clear plastic cup, a metal clip, and white plastic mesh; the cage measures 30 mm in diameter, 30 mm in height and 5 g in weight, and covers a leaf area of approximately 7 cm^2 (Zang *et al.*, 2005c). Five days after the adults were introduced into the clip-cages, they were removed and all eggs produced were allowed to develop on the caged leaves. As the progeny developed to adults and emerged, they were collected and sexed, and their biotype identity was determined by RAPD-PCR. The proportions of females in F1 progeny in the four intra-biotype crossing treatments were analyzed using one-way ANOVA, followed by Tukey test of multiple comparisons.

Observation on Mating Behaviour and the Timing of First Mating

Newly-eclosed virgin adults were introduced in a Plexiglas cell (Fig. 1) for observation. A cotton leaf was placed at the bottom of the Plexiglas observation cell (Fig. 1), which enclosed approximately 7 cm^2 leaf area. There

Fig. 1. Plexiglas cell used to observe mating behaviour of *Bemisia tabaci*.

were four treatments, with ca. 20 replicates in each treatment (Table II). Virgin adults were collected between 07:00–08:00 (i.e., 1–2 h after the onset of the photophase). One female and one or two males in each replicate were then introduced into a cell, and observation was done through a clear glass cover. The behaviour of adults in each replicate was then continuously recorded for 12 h under constant fluorescent lighting using a video camera (SONY DCR-HC40E, Japan). The light intensity was 500 lux at the leaf surface within the observation cell. The mating behaviour, starting from when the male began to search for a female (see definition below) to the end of copulation was observed and recorded through replay of the video recording. Time spent at each phase in the course of mating was determined using the time recorder in the video camera (Table III). The frequencies of first copulation in each time interval between various combinations of males and females were compared by an $R \times C$ contingency table using G-test. The durations of time intervals for various behavioural components between treatments were analyzed by a two-way ANOVA with biotype and the number of males as the two experimental factors.

Table II. Number of Replicates of Various Combinations of Males and Females of B Biotype or ZHJ1 of *Bemisia tabaci* in Which the First Copulation Occurred during Various Time Intervals after Emergence

Treatments	Total no. of replicates	No. of replicates in which the first copulation occurred between various intervals from 4–12 h post emergence				
		4–6 (h)	6–8 (h)	8–10 (h)	10–12 (h)	Total
1 B ♀ × 1 B ♂	22	2	4	4	3	13
1 B ♀ × 2 B ♂	20	1	5	6	2	14
1 ZHJ1 ♀ × 1 ZHJ1 ♂	21	3	5	2	4	14
1 ZHJ1 ♀ × 2 ZHJ1 ♂	20	5	6	3	1	15

Observation on Frequency of Copulation

The number of copulation events in the first 72 h after emergence was investigated by recording these behaviours using SONY video cameras day and night. We used illumination provided by a 15 watt ordinary red light to enable recording by the video camera during nighttime. Events were recorded on the video tape for 2 s every 30 s throughout the 72 h by using the function of interval recording of the camera. This frequency of recording was applied to catch every copulation event as detailed observations on mating behaviour showed that a complete copulation event never lasted less than 90 s. The data were analyzed using one-way ANOVA, followed by Tukey test of multiple comparisons (Table IV).

Observation on Events for Females being Courted by Males

Newly-eclosed virgin adults were placed on cotton leaves enclosed by clip-cages at 08:00. There were four treatments (Table V), each with 20

Table III. Duration of Different Phases in the Mating Behaviour of the B Biotype and ZHJ1 of *Bemisia tabaci*

Biotype	Treatments	No. of replicates	Mean (±SE) searching time (s)	Mean (±SE) courting time (s)	Mean (±SE) copulating time (s)
B	1 ♀ × 1 ♂	13	274 ± 65 a	415 ± 50 a	136 ± 6 b
	1 ♀ × 2 ♂	14	319 ± 78 a	456 ± 94 a	137 ± 5 b
ZHJ1	1 ♀ × 1 ♂	13	363 ± 52 a	355 ± 55 a	171 ± 7 a
	1 ♀ × 2 ♂	11	225 ± 51 a	485 ± 102 a	150 ± 5 b

Note. Means of the same biotype in the same column followed by the same letter do not differ significantly ($P > 0.05$).

Table IV. Number of Copulation Events Observed in the First 72 h Post-emergence in Various Combinations of Males and Females of B biotype and/or ZHJ1 of *Bemisia tabaci*

Treatments	No. of Replicates	Mean number (±SE) of copulation[a]
1 B ♀ × 1 B ♂	6	1.5 ± 0.2 b
1 B ♀ × 2 B ♂	5	4.2 ± 0.6 a
1 ZHJ1 ♀ × 1 ZHJ1 ♂	5	1.0 ± 0.0 b
1 ZHJ1 ♀ × 2 ZHJ1 ♂	5	1.2 ± 0.2 b
1 B ♀ × 2 ZHJ1 ♂	6	0
1 ZHJ1 ♀ × 2 B ♂	6	0

[a] Means in this column followed by the same letters do not differ significantly ($P > 0.05$). Data of the two inter-biotype crosses were not included in the ANOVA because of no copulation.

replicates. Adults in each replicate were observed once every 2 h from 08:00 to 20:00 each day, i.e., seven times per day. The observation was conducted for five days. Whenever a male and a female were found close to each other with their bodies lined up (see Fig. 2), it was recorded as an event of the female being courted by the male. Courtship between a female and a male is a necessary prelude to copulation (though courtship does not always result in copulation), and thus frequency of courtship indicates the propensity to mate. The courtship events recorded were used to estimate the probability of courtship between the two sexes using the following formula: Probability of courtship = (number of courtship events)/(total number of observations). The probability data were transformed by arcsine square root and analyzed by a two-way ANOVA, with biotype and the number of males as the two experimental factors.

Table V. Probability of Courtship in Various Combinations of Males and Females of B biotype or ZHJ1 of *Bemisia tabaci* during a Period of Five Days

Biotype	Treatment[a]	Mean probability (±SE) of courtship[b]
B	1 ♀ × 1 ♂	0.27 ± 0.03 B
	1 ♀ × 2 ♂	0.53 ± 0.03 A
ZHJ1	1 ♀ × 1 ♂	0.20 ± 0.03 a
	1 ♀ × 2 ♂	0.18 ± 0.04 a

[a] Twenty replicates in each treatment.
[b] Means of the same biotype in the same column followed by different letters differ at $P < 0.05$ (small letters) or $P < 0.001$ (capital letters) level.

Fig. 2. Courtship of one pair (♂ on the left) of *Bemisia tabaci* on a leaf.

RESULTS

Progeny of Crosses

No females were produced from crosses between B and ZHJ1, while all the four intra-biotype crosses produced both female and male progeny (Table I). In the control crosses within the B biotype, the proportion of female progeny in the "1 B ♀ × 2 B ♂" treatment was significantly higher than that in the "1 B ♀ × 1 B ♂" treatment. However, the proportions of females in F1 progeny in the two control crosses within ZHJ1 were similar to each other, and were also similar to that in the "1 B ♀ × 1 B ♂" treatment (Table I).

Mating Behaviour

In all, 504 h of video recording from 42 females and 62 males of the B biotype and 492 h of video recording from 41 females and 61 males of ZHJ1 were replayed and observed. These video recordings recorded 68 copulation events and showed that both biotypes exhibited very sophisticated courtship and mating behaviour. With our video equipment, we were unable to document many details of the behaviour. However, for a gross comparison between the two biotypes, the courtship behaviour leading to

successful copulation in both biotypes can be described as three consecutive phases:

Phase 1: Male search for female. Before sexual encounter, almost all males and females feed on the leaves and exhibit little motion. At some stage, the male becomes active, and begins to move around on the leaf. It usually takes the male 3–8 min to encounter a female. The time interval from the point when the male becomes active to the point when the male first contacts a female is termed the "searching time."

Phase 2: Courtship. In this phase, the male positions himself parallel to the female, and then uses one of his antennae to touch one antenna of the female (Fig. 2). Antennal touch and drumming may last for only part of the time during courtship. The time interval from the point when the male makes physical contacts with a female to the point when copulation starts to take place is termed "courting time". Courting usually lasts for 6–10 min.

Phase 3: Copulation. The male initiates copulation by positioning his abdomen below that of the female, at a 30–45° horizontal angle to her abdomen. The male raises all four wings to cover the female's wings, though the pair of wings of the male further away from the female often do not overlap completely with those of the female. During copulation, the male vibrates his wings at high frequency. Copulation ends with the separation of abdomens between the male and female. The time interval from initiation to the end of copulation is termed the "copulating time." Copulation usually lasts for 2–4 min.

It was noted that, in many cases, the sequence of mating behaviour terminated at some stage of Phase 2, that is, courtship frequently did not lead to successful copulation.

Timing of First Mating

A few females of both biotypes accepted courting males and copulated with them between 4–6 h after emergence and over half the adults of both biotypes completed their first copulation in the first 12 h after emergence (Table II). The proportions of females that mated in the first 12 h did not differ between B biotype and ZHJ1 ($\chi^2 = 4.18$; df = 4; $P > 0.05$). The proportion of females that mated in the first 12 h was not influenced by the addition of one extra male (For the B biotype: $\chi^2 = 1.55$, df = 4, $P > 0.05$; for ZHJ1: $\chi^2 = 2.90$, df = 4, $P > 0.05$).

Duration of Behavioural Components

We compared only those events that led to successful copulation. There was no significant difference in the duration of searching time

between the two biotypes (Table III. $F = 0.234$, df = 1, 47; $P > 0.05$). Likewise, there was no significant difference in the duration of courting time between the two biotypes (Table III. $F = 0.154$; df = 1, 47; $P > 0.05$). However, the mean copulating time of ZHJ1 in the "1 ♀ × 1 ♂" treatment was significantly longer than that of ZHJ1 in the "1 ♀ × 2 ♂" treatment as well as those in the two treatments of the B biotype ($F = 17.489$; df = 1, 47; $P < 0.001$). The durations of the three behavioural components of B biotype were not significantly influenced by the addition of one extra male. However, the duration of copulating time of ZHJ1 was significantly decreased by the addition of one extra male (Table III).

Frequency of Mating

When males and females of the same biotype were placed together, courtship behaviour and copulation were always observed. However, males and females from the inter-biotype crosses frequently exhibited courtship behaviour but never successfully copulated (Table IV). Males and females could mate more than once, especially those in the B biotype. The mean number of mating events of "1 B ♀ × 1 B ♂" treatment was marginally higher than that of "1 ZHJ1 ♀ × 1 ZHJ1 ♂" treatment (P = 0.06; Table IV). Within the B biotype, the mean number of copulation events in the "1 ♀ × 2 ♂" treatment was significantly higher than that of "1 ♀ × 1 ♂" treatment. However, within ZHJ1, the "1 ♀ × 2 ♂" treatment had no greater number of copulation events than that of the "1 ♀ × 1 ♂" treatment.

Probability of Courtship

The probability of courtship between females and males in the B biotype during the 5-day period was significantly higher than that in ZHJ1 ($F = 22.43$; df = 1, 76; $P < 0.001$). In the B biotype, the probability of courtship in the "1 B ♀ × 2 B ♂" treatment was significantly higher than that in the "1 B ♀ × 1 B ♂" treatment. However, for ZHJ1, the addition of one more male did not increase the probability of courtship (Table V).

DISCUSSION

Reciprocal crosses between the B biotype and ZHJ1 did not produce any female progeny and thus indicated reproductive incompatibility between the two biotypes (Table I). Whiteflies exhibit haplo-diploidy,

producing males from unfertilized eggs and females from fertilized eggs (Byrne and Bellows, 1991). The lack of female progeny can have several causes: (i) no insemination takes place, (ii) insemination takes place but males develop from fertilized eggs, (iii) fertilized eggs die and all male offspring produced stem from unfertilized eggs (Stouthamer *et al.*, 1996). As the opposite sexes of two biotypes placed together did not mate (Table IV), the lack of hybrid female offspring in F1 resulted from no insemination. This complete reproductive incompatibility is similar to that reported for biotypes A × B (Perring *et al.*, 1993), and to that for biotypes B × K, B × M, B × D, K × M, K × D, and M × D (Bedford *et al.*, 1994). Interestingly, De Barro and Hart (2000) reported that the B biotype and either of two Australian biotypes (EAN and WAN) when placed together produced F1 hybrid females, although the hybrid females were most probably sterile. Hybrid females have also been reported for crosses between biotypes B × L (Byrne *et al.*, 1995), between biotypes B × Q (Ronda *et al.*, 2000), and between two genotypes from the Sub-Saharan Africa and the unresolved Asia group (Maruthi *et al.*, 2001; see De Barro *et al.*, 2005). As De Barro *et al.* (2005) commented, the data so far indicate that crosses between individuals representing the major races, as defined by them, resulted in males only, while crosses between individuals belonging to subraces within the same major race may produce female progeny.

Our research indicates that there are significant differences in mating behaviour between B and ZHJ1. The two biotypes exhibited similar durations of searching time and courting time in the mating process. However, the copulating time of ZHJ1 was longer than that of B biotype. Interestingly, ZHJ1 showed a significant reduction in copulation time when an additional male of same biotype was added. In contrast, there was no similar effect in the B biotype (Table III). More importantly, the frequency of mating in the B biotype was marginally higher than that in ZHJ1 (Table IV). Moreover, the frequency of mating by females in the B biotype increased by nearly three fold when an extra male of the same biotype was added, while addition of an extra male in the ZHJ1 did not result in increase of mating frequency (Table IV). These results indicate that individuals of the B biotype have a higher propensity to mate than those of ZHJ1 when the proportion of males is increased in the colony. These differences in propensity of mating between the two biotypes were also reflected by the data of observations on courtship frequency (Table V). More detailed differences in courtship and copulation behaviour between these two biotypes may be revealed by video recording with higher resolution (e.g., Perring and Symmes, 2006) than that used in the current study. It is likely that differences in mating behaviour play a critical role in preventing copulation between B biotype and ZHJ1 of *B. tabaci* and between other biotypes of

this species complex (Perring *et al.*, 1993; Butlin, 1995; Perring and Symmes, 2006).

Reproductive interference and competitions between the B biotype and other biotypes have been demonstrated. Perring *et al.* (1993) and Perring (1996) indicated that males of the B biotype had longer time of courtship than did those of the A biotype, and they could block courtship and mating between males and females in the A biotype. De Barro and Hart (2000), Pascual and Callejas (2004) and De Barro *et al.* (2006) showed that the proportion of biotype B individuals in mixed cultures with other non-B biotypes increased over one to three generations to a point where the B biotype dominated the population. Zang *et al.* (2005b) demonstrated that the non-B ZHJ1 population was completely displaced by the B biotype after 6 generations when they were initially placed together in equal numbers on a mutually acceptable host. Differential female fecundity is one of the mechanisms of competition that can lead to displacement of one population by another (Reitz and Trumble, 2002). The mean number of copulation events for a B biotype female increased from 1.5 to 4.2 with the presence of an additional male (Table IV). Corresponding proportion of females in progeny increased from 0.44 to 0.74 (Table I). However, the mean number of copulation events and proportion of females in progeny show no similar increases for ZHJ1 (Tables I and IV). The increase in mating activity with additional males in the B biotype resulted in a higher female-biased sex ratio in progeny. These data indicate a higher reproductive potential in the B biotype compared to ZHJ1 population, which may be one of the major factors responsible for the displacement of ZHJ1 population by B biotype (Zang *et al.*, 2005b). Presently, the B biotype, a successful invader, has spread rapidly around the world to become a considerable pest of agriculture (Brown *et al.*, 1995). Factors responsible for successful invasion of the B biotype include competing for ecological niche (Perring, 1996), reproductive interference (Perring, 1996; De Barro and Hart, 2000; Pascual and Callejas, 2004; De Barro *et al.*, 2006), differences in host plant adaptation (Brown *et al.*, 1995; Zang *et al.*, 2006), insecticide resistance (Byrne and Devonshire, 1993; Riley and Tan, 2002) and association with whitefly-transmitted geminivirues (Mayer *et al.*, 2002; McKenzie *et al.*, 2002) between biotypes. The results obtained here suggest that the B biotype has some advantage in adjusting sex ratio across generations in responding to environmental factors, a feature that may be important to make the B biotype a good invader.

In the present study, some females of both biotypes began to mate 4–6 h after emergence, and over half of them completed the first copulation within 12 h after emergence (Table II). Byrne *et al.* (1995) also demonstrated that some adults of *B. tabaci* biotypes B and L mated within 6 h

of emergence at 27°C. Avidov (1956, cited in Byrne and Bellows, 1991) reported that *B. tabaci* from Israel mated within 1–8 h following eclosion from pupal case during summer months. Li *et al.* (1989) reported that *B. tabaci* biotype A females did not start to mate until 20–24 h after emergence, and this information on the timing of first mating has been used to design procedures for collecting virgin adults in some subsequent studies with other biotypes (Perring *et al.*, 1993; De Barro and Hart, 2000; Maruthi *et al.*, 2001; Perring and Symmes, 2006). The apparent differences in the timing of first mating between the two biotypes of *B. tabaci* in the current study and biotype A in Li *et al.* (1989) may be due to different biotypes or different rearing facilities and procedures in the two studies. Information on the timing of the first mating for the biotypes or populations under study is critical in developing appropriate procedures for collecting virgin adults in mating and other experiments.

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